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Fine-root respiration in a loblolly pine (*Pinus taeda* L.) forest exposed to elevated CO_2 and N fertilization

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ABSTRACT

Forest ecosystems release large amounts of carbon to the atmosphere from fine-root respiration (R_r), but the control of this flux and its temperature sensitivity (Q_{10}) are poorly understood. We attempted to: (1) identify the factors limiting this flux using additions of glucose and an electron transport uncoupler (carbonyl cyanide m-chlorophenylhydrazone); and (2) improve yearly estimates of R_r by directly measuring its Q_{10} *in situ* using temperature-controlled cuvettes buried around intact, attached roots. The proximal limits of R_r of loblolly pine (*Pinus taeda* L.) trees exposed to free-air CO_2 enrichment (FACE) and N fertilization were seasonally variable; enzyme capacity limited R_r in the winter, and a combination of substrate supply and adenylate availability limited R_r in summer months. The limiting factors of R_r were not affected by elevated CO_2 or N fertilization. Elevated CO_2 increased annual stand-level R_r by 34% whereas the combination of elevated CO_2 and N fertilization reduced R_r by 40%. Measurements of *in situ* R_r with high temporal resolution detected diel patterns that were correlated with canopy photosynthesis with a lag of 1 d or less as measured by eddy covariance, indicating a dynamic link between canopy photosynthesis and root respiration. These results suggest that R_r is coupled to daily canopy photosynthesis and increases with carbon allocation below ground.

Key-words: carbon cycle; Duke FACE; FACTS-1; global climate change; net ecosystem exchange; soil respiration.

INTRODUCTION

Plant (autotrophic) respiration (R_a) is globally important and releases about 60 Gt C to the atmosphere each year (Prentice *et al.* 2001), roughly eight times the flux of C from fossil fuels (7.2 Gt C year^{-1} from 2000 to 2005; IPCC 2007). As forest ecosystems comprise the largest portion of the terrestrial C flux (Prentice *et al.* 2001), small changes in R_a from forests could have a large effect on the global carbon cycle. Although R_a is typically predicted to increase with climate change because of its positive correlation with temperature (Boone *et al.* 1998; Friedlingstein *et al.* 2006), ecosystem responses to global change factors can influence the amount of respiring biomass or its temperature sensitivity, leading to a more complex relationship between global change and R_a (Luo 2007). Thus, it is important to understand how major aspects of global change such as increases in atmospheric CO_2 (IPCC 2007) and nitrogen deposition (Galloway *et al.* 1995) affect R_a in forest ecosystems.

Because of the lack of a rigorous mechanistic model equivalent to that for photosynthesis (Farquhar, Caemmerer & Berry 1980), carbon cycling models are forced to make simplifying assumptions to incorporate R_a. Whereas some models assume R_a is a constant fraction of gross primary production (DeLucia *et al.* 2007), many physiological models (Aber & Federer 1992; Thornton *et al.* 2002) make the assumption that R_a increases exponentially with temperature with a constant Q_{10} of ~2 (Q_{10}: multiplicative change in R_a with a 10 °C change in temperature). However, the Q_{10} of vegetation varies by species, tissue type, temperature and environmental conditions (Tjoelker, Reich & Oleksyn 1999; Atkin, Holly & Ball 2000; Atkin *et al.* 2005; Bernhardt *et al.* 2006), with substantial impacts ecosystem carbon cycling. For example, allowing Q_{10} to acclimate to air temperature reduced modeled leaf respiration by 31–41% and increased above-ground net primary production by 18–38% in a boreal coniferous forest (Wythers *et al.* 2005).

Despite its importance, few field studies have estimated the Q_{10} of below-ground processes directly. Some studies have used seasonal changes in temperature to develop a temperature function for respiration (Lloyd & Taylor 1994; Zha *et al.* 2004; Rodeghiero & Cescatti 2005), but this method confounds other variables with temperature (e.g. phenology), and is not capable of detecting seasonality in Q_{10} (Davidson, Janssens & Luo 2006). One objective of this study was to improve annual estimates of fine-root respiration (R_r) in a loblolly pine (*Pinus taeda* L.) forest by directly measuring the temperature dependence of respiration (Q_{10}) throughout the year. Fine-root respiration was investigated because it is the largest component of R_a in this ecosystem, comprising ~40% of the total flux (Hamilton *et al.* 2002).

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Atkin & Tjoelker (2003) proposed a tripartite mechanistic model of regulation, where R_a is proximally limited by enzyme capacity, substrate availability or negative feedbacks on the tricarboxylic acid (TCA) cycle by ATP production (i.e. limited by adenylate availability). Enzyme capacity generally limits R_a at low temperatures (Covey-Crump, Attwood & Atkin 2002), whereas substrate or adenylate limitations are common at moderate to high temperatures (Noguchi & Terashima 1997; Covey-Crump *et al.* 2002). The realized rate of respiration is determined by the minimum of these limiting factors.

To our knowledge, this model of respiratory control has not been applied in the field or on trees where the seasonality of C allocation and elements of global change could alter these proximal limits of R_a. We hypothesize that elevated atmospheric CO_2 will alleviate substrate limitation of root respiration (R_r) by increasing tissue carbohydrate supply (Ainsworth & Long 2005), wheras simulated nitrogen deposition will increase respiratory capacity by providing more N for protein synthesis. Understanding how global change influences the limitations of R_a would lend confidence to future predictions of this important flux.

This study had two specific objectives: (1) identify the limits of fine-root respiration (R_r) of loblolly pine trees over a seasonal cycle, and to investigate modifications of these limitations by N fertilization and elevated CO_2; and (2) improve annual estimates of R_r by directly measuring the Q_{10} multiple times throughout the season.

METHODS

Site description

This research was conducted at the Duke free-air carbon dioxide enrichment (FACE) experiment (Orange County, NC, USA; 35°58′N 79°05′W) comprised of six 30-m-diameter plots within a continuous, unmanaged loblolly pine (*Pinus taeda*) plantation. Three fully instrumented control plots receive ambient air, and three treatment plots maintain atmospheric CO_2 concentration at ambient plus 200 μmol mol^{-1} to simulate conditions expected in the year 2050 (IPCC 2007). The experimental design has been expanded to include the FACE prototype and reference plots, but this study was conducted in the original six plots only. In 2006, the year of this study, average daytime CO_2 concentration was ~383 μmol mol^{-1} in the ambient plots, and ~577 μmol mol^{-1} in the elevated plots. The CO_2 concentrations at night were similar in both treatment and control plots (~410 μmol mol^{-1}; Keith Lewin, Robert Nettles personal communication). Soils are of the Enon Series derived from mafic bedrock (fine, mixed, active, thermic Ultic Hapludalfs) and are slightly acidic (0.1 M $CaCl_2$ pH 5.5). Detailed descriptions of the FACE technology (Hendrey *et al.* 1999) and soils at this site are available (Oh & Richter 2005).

A nitrogen fertilizer treatment was added to the FACE experiment in 2005. Each year, ammonium nitrate was hand-broadcasted to half of each plot at a rate of 11.2 gN m^{-2} year^{-1} in two applications (half in March, half in April). The unfertilized half of each plot was separated from this treatment by a 70-cm-deep impenetrable tarp; 95% of fine roots are <15 cm deep and nearly 0% are >30 cm (Matamala & Schlesinger 2000). The experimental design at the time of this study was a split-plot in a randomized complete block design with three replicates; CO_2 treatment was the whole-plot factor and N treatment was the subplot factor.

Net ecosystem exchange of CO_2 (NEE) was measured with an eddy covariance system (EC) comprised of a triaxial sonic anemometer (CSAT3, Campbell Scientific, Logan, UT, USA) coupled with an open-path infrared gas analyzer (LI-7500, Li-Cor, Lincoln, NE, USA) positioned 20.2 m above an upwind ambient CO_2 plot. The Webb–Pearman–Leuning correction for the effects of air density fluctuations on flux measurements was applied to scalar fluxes measurements (Webb, Pearman & Leuning 1980), and a 1/2 h averaging interval was chosen. More information about these measurements and subsequent data analyses are available (Katul *et al.* 1997; Stoy *et al.* 2006a,b).

Oxygen electrode measurements

The rate of oxygen consumption before and after the addition of exogenous glucose or an electron transport uncoupler (carbonyl cyanide m-chlorophenylhydrazone, CCCP) was measured on excised roots three times during the year. Two subsamples were averaged per subplot in May and three subsamples were averaged per subplot in July and January. These sampling dates were chosen to capture variation in C allocation, as maximum wood growth is in May (Moore *et al.* 2006), needle and fine-root growth peaks are in July (Schafer *et al.* 2003), and little growth occurs in January. Roots were sampled between 0900 and 1200 h to minimize potential time-of-day effects. Attached *P. taeda* fine roots (≤1.5 mm diameter) were excavated by removing the litter layer and gradually exposing roots with paint brushes. Roots were excised with a razor blade and stored in 1 mM $CaCl_2$ buffered to pH 5.50 with 2-Morpholinoethanesulfonic acid (MES) during transport to an on-site laboratory (~5 min). Approximately 0.5 g fresh weight (FW) of fine roots were cut into 3 cm segments and divided into three subsamples. One subsample was stored in liquid N_2 for analysis of carbohydrates. The other subsamples were incubated for 20 min in buffer or buffer plus glucose (50 mM) at a controlled temperature that approximated ambient soil temperature (20 °C in May and July, 10 °C in January). Increased R_r in the glucose-saturated sample relative to the subsample without added glucose (hereafter the 'basal sample') would indicate that the availability of sugar substrates limited basal R_r.

Respiration of the paired subsamples (basal and glucose-saturated) was measured concurrently at incubation temperature in Clark-type oxygen electrodes (Dual Digital Model 20; Rank Brothers, Cambridge, UK). The respiration rate was measured over a period of 10 min following 5 min of equilibrium in the electrode. CCCP was then injected to

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the basal sample to a final concentration of 15 μL and the uncoupled respiration rate was measured over the next 10 min following a 10 min equilibration period. CCCP dissipates the H^+ gradient across the inner mitochondrial membrane, uncoupling proton transport from ATP synthesis. An increase in R_a upon addition of CCCP would indicate that the H^+ gradient across the mitochondrial membrane limited O_2-consumption (Lambers, Robinson & Ribas-Carbo 2005; Papa, Lorusso & Di Paola 2006). All measurements were completed <90 min after root excision. Rates of oxygen consumption were converted to CO_2 efflux to facilitate comparisons with gas exchange measurements assuming a respiratory quotient of 1.25 (Penning de Vries, Brunsting & Van Laar 1974; Matamala & Schlesinger 2000). One measurement was taken per subplot of a single block per day. The concentrations of glucose and CCCP used in this study saturated the stimulation of O_2 consumption in these fine roots (Drake, unpublished).

Tissue chemistry

Each frozen root subsample was ground using mortar and pestle in liquid N_2 and immediately subjected to three extractions in 80% ethanol and 2 mM Hepes (pH 7.8), and one extraction in 50% ethanol and 2 mM Hepes (pH 7.8; all at 80 °C for 20 min). Concentrations of glucose, fructose and sucrose were quantified spectrophotometrically (Jones, Outlaw & Lowry 1977; Hendrix 1993) at 340 nm with a 96 well plate reader (Powerwave HT; Biotek, Winooski, VT, USA). Starch was degraded to glucose by overnight incubation with amyloclucosidase and α-amylase at 37 °C, and quantified as glucose equivalents in the plate reader. The other root subsamples were dried and combusted in an elemental analyzer to determine C and N contents (ECS 4010; Costech, Valencia, CA, USA).

Gas exchange measurements

The *in situ* rate of CO_2 evolution was measured in July and January by enclosing intact, attached fine roots in buried gas exchange cuvettes. About 0.3 g FW of attached fine root tissue was excavated from 0 to 5 cm below the litter layer, washed as described previously, patted dry and placed into a custom polycarbonate cuvette with a type-E thermocouple. The leaf litter and disturbed soil was replaced to allow the cuvettes to reach thermal equilibrium with the soil. Four cuvettes were prepared in this way per subplot.

Root CO_2 efflux was measured using a custom open-path automated sampling system built around a closed-path infrared gas analyzer (Li 6262; Li-Cor). Ambient air that passed through two 122 L buffer volumes was used as the input gas in the ambient plots; high temporal variation in [CO_2] necessitated the use of standard air tanks (400 μmol CO_2 mol^{-1}) in elevated CO_2 plots. Air was humidified as much as possible (~80% relative humidity) using a series of water bubblers and traps. Gas manifolds containing five solenoid valves (Mac Valves, Wixom, MI, USA) allowed the air flow to be directed to a reference line or one of four cuvettes. The system was controlled by a data logger (CR10X; Campbell Scientific, Logan, UT, USA). The flow rate was measured with a mass flow meter (Hastings ST-1K; Teledyne Hastings, Hampton, VA, USA) upstream of the manifolds. A single measurement consisted of passing air through a cuvette for 4 min and then through the reference line for 1 min. The data logger recorded 10 s averages of cuvette temperatures and CO_2 concentration and computed a difference measurement as $\Delta CO_2 = [CO_s]_{cuvette} - [CO_2]_{reference}$ (Long *et al.* 1993). A measurement cycle of all four chambers was achieved every 20 min. A 24 h diel cycle of R_r was measured on all sampling dates after roots acclimated to the cuvettes for 5 h. Q_{10} values were calculated from diel variation according to the following equation: $Q_{10} = (R_2/R_1)^{[10(T_2-T_1)]}$; where T_1 and R_1 denote temperature and R_r at the daily minimum temperature (0600 to 0800 h), and T_2 and R_2 denote temperature and R_r at the subsequent maximum temperature (1300 to 1500 h). Soil temperature and R_r were relatively constant between 0400 and 0700 h; values during this period were averaged to calculate basal R_r for each plot.

Temperature response of R_r

The temperature sensitivity of respiration (Q_{10}) was measured following 24 h of *in situ* measurements by modulating cuvette temperatures with an external water bath that circulated water through the base of each cuvette. R_r was measured at five temperatures from 5 to 40 °C; two measurements per cuvette were averaged per temperature. Roots were excised, dried and analyzed for C and N content as described previously. Subplots of each main plot were sampled on successive days. Sampling of all plots was completed over 2 weeks in July 2006 and January 2007. The temperature response was not measured in N-fertilized subplots because of time constraints.

Scaling R_r to the stand-level

The basal *in situ* respiration rates were scaled to yearly estimates using soil temperature at a depth of 10 cm, the measured temperature sensitivity of R_r and plot-specific measurements of fine-root biomass. Fine-root biomass was measured every 3 months with soil cores (4.75 cm diameter, 15 cm deep, $n = 3$ per subplot). Roots were picked by hand, dried and weighed (Jackson, unpublished). Monthly fine-root biomass was estimated by interpolating plot averages with a linear spline function (Proc Expand, SAS v9.1; SAS Institute, Cary, NC, USA). In 2006, annually averaged fine-root biomass in ambient $CO_2 \times$ ambient N, elevated $CO_2 \times$ ambient N, ambient $CO_2 \times$ N fertilized and elevated $CO_2 \times$ N fertilized were 250, 374, 291 and 347 g m^{-2}, respectively (data not shown). Thirty-minute averages of soil temperature were measured at 10 cm depth in four locations in the ambient plot associated with the eddy covariance system. These measurements were averaged by month to

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coincide with the biomass estimates. Values of R_r measured *in situ* were converted to monthly estimates using the measured temperature response. The summer rates and temperature response were used for May–November whereas the winter values were used for December–April, following the growing and dormant seasons at this site (Moore *et al.* 2006). Applying these rates and functions to different combinations of months altered the yearly R_r estimates by less than 5%.

Data analysis

Statistical analyses followed a repeated measures split-plot design and were computed using SAS (v9.1; SAS Institute). Repeated-measures mixed-model analyses of variance (Proc Mixed) were used in all analyses except for the regressions and temperature-response curves, where least squares regressions were used (Proc Reg). The apparent Q_{10} curves were fit in Sigmaplot 10.0 (Systat, San Jose, CA, USA). The lag analysis was performed as in Ekblad & Hogberg (2001). Covariance structures in the repeated-measures analyses were modeled as autoregessive-1, as this minimized the fit statistics based on the -2 res log likelihood parameter (Littell, Henry & Ammerman 1998). All analyses were checked to ensure homoscedasticity and normality of residuals; transformations were applied where appropriate. Unless otherwise stated, all data are presented as least-squares means and error bars are ±1 SE as estimated within a mixed model [i.e. least squares (LS)–standard errors].

RESULTS

Respiratory control and tissue chemistry

Low rates of R_r and the absence of a response to exogenous glucose or CCCP suggest that R_r was limited by enzyme capacity during January (Fig. 1). In contrast, R_r was limited by a combination of substrate supply and ATP utilization during May and July, as R_r was significantly increased by the addition of glucose and CCCP. There were no significant main effects or interactions of elevated CO_2 or N on R_r, or its stimulation by substrate or uncoupler ($P > 0.2$), so the LS means of sampling date were presented for clarity (Fig. 1). Uncoupler stimulated R_r by 31.0 and 16.6% in May and July, whereas glucose additions stimulated R_r by 21.6 and 19.9%, respectively.

Fine root carbohydrate contents were seasonally variable (Table 1). Concentrations of glucose increased in the winter, whereas fructose concentrations decreased. Starch decreased in July, the period of maximal root production (Pritchard *et al.* 2008), suggesting that this starch was used for growth. Notably, there were no direct effects of elevated CO_2 or N fertilization on fine-root carbohydrates. Nitrogen fertilization caused an average 22.8% increase in fine-root N but did not increase R_r.

Fine-root O_2 consumption rates were positively correlated with tissue sucrose and N concentrations, but the degree of substrate or adenylate restriction was not related

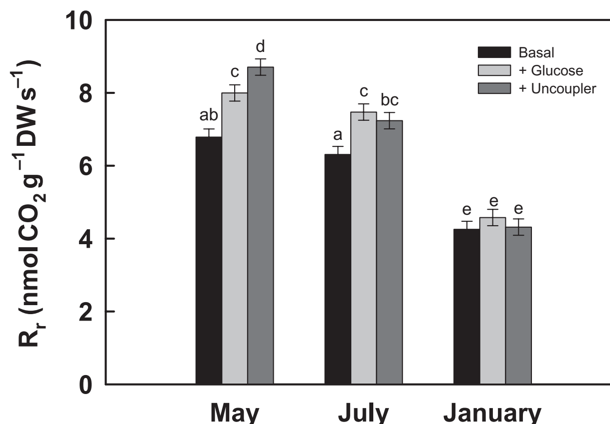

Figure 1. Basal respiration (R_r) of excised fine roots and the rates following addition of glucose or a mitochondrial uncoupler (carbonyl cyanide m-chlorophenylhydrazone) in liquid-phase oxygen electrodes assuming a respiratory quotient of 1.25. Categories that do not share a letter are significantly different (Tukey adjusted $P < 0.05$). Measurement temperatures approximated soil temperatures: 20 °C in May and July and 10 °C in January.

to any measured aspect of tissue chemistry. Sucrose was positively correlated with basal, glucose-saturated and CCCP-uncoupled respiration rates (data not shown, log–log plots, respective slopes = 0.027, 0.031, 0.036; respective $r^2 = 0.29$, 0.26, 0.32; $P < 0.01$), but sucrose concentrations could not explain the differences between these rates. Root N content was positively correlated with basal respiration rate, but the slope was significantly decreased by N fertilization [analysis of covariance (ANCOVA), data not shown, log-log plots, slope in ambient N = 0.086 ± 0.003; N-fertilized = 0.0718 ± 0.003, $r^2 = 0.26$ and 0.12, respectively, ANCOVA $P < 0.01$].

In situ R_r: CO_2 efflux

Basal R_r measured by gas exchange on attached roots in the field (Fig. 2) varied with sampling date ($P < 0.01$), and was reduced by the combination of elevated CO_2 and N fertilization in July (Tukey adjusted $P < 0.05$). No treatment effects were observed in January ($P > 0.5$). Averaged across treatments, R_r was 8.60 and 1.87 nmol CO_2 g^{-1} dry weight (DW) s^{-1} in July and January, respectively.

Temperature sensitivity of R_r

The relationship between R_r and temperature (Fig. 3a) was best described by a linear regression, although a small but significant second-order term was present in July (July: $y = -7.25 + 1.17x - 0.011x^2$, $P < 0.01$, $r^2 = 0.61$; January: $y = -0.0115 + 0.173x$, $P < 0.01$, $r^2 = 0.68$). The observed temperature sensitivity of R_r (slope) was significantly higher in July than in January (ANCOVA, $P < 0.01$). The observed data could only be described by the traditional exponential function if the Q_{10} declined with temperature (Fig. 3b). There

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Table 1. Chemistry of *Pinus taeda* fine roots grown in the field under elevated CO_2 and N fertilization

CO_2	N	Month	Glucose	Fructose	Sucrose	TSC	Starch	C %	N %	C : N
Control	Control	May	3.6	7.8	21.7	33.2	64.0	50.9	1.22	43.2
		July	3.8	6.1	16.0	25.9	42.9	51.7	1.20	43.5
		January	8.7	1.6	10.7	21.0	62.5	51.7	1.11	47.1
Control	Fertilized	May	1.9	8.4	21.0	31.3	60.4	52.0	1.64	32.4
		July	3.3	4.8	15.2	23.3	41.4	51.9	1.46	35.8
		January	6.8	1.4	8.5	16.7	59.5	51.9	1.38	38.2
Elevated	Control	May	2.5	3.6	11.5	17.5	72.0	50.8	1.17	43.7
		July	3.7	6.1	17.4	27.2	29.2	52.1	1.18	44.7
		January	8.9	1.6	11.3	21.8	46.7	51.8	1.06	49.1
Elevated	Fertilized	May	4.8	8.1	18.1	31.0	89.1	52.3	1.45	37.1
		July	2.9	7.0	17.7	27.6	12.9	52.7	1.38	38.6
		January	8.5	1.4	10.8	20.7	55.7	51.9	1.21	43.3
Significant effects			D	D, D × C	D	D	D	N	D, N	D, C, N

Statistically significant main effects and interactions ($P < 0.05$) are shown in the bottom row.
Least-squares means are shown by treatment and sampling date.
Standard errors estimated from repeated-measures mixed-model analyses of variance are as follows: glucose, 0.9; fructose, 0.9; sucrose, 2.5; total soluble carbohydrates (TSC; glucose + fructose + sucrose), 3.7; starch, 19.4, C %, 0.6; N %, 0.05, C : N, 1.4.
Units for glucose, fructose, sucrose and TSC are μmol g^{-1} DW. Starch values are in μmol glucose equivalents g^{-1} DW.
D = sampling date, C = CO_2 treatment, N = nitrogen treatment (interactions are shown as combinations of these letters).

was no observable difference in the temperature sensitivity of respiration by roots grown at different CO_2 concentrations (ANCOVA, $P > 0.4$).

Diel R_r variation

Diel variation in R_r was relatively small in the winter, but large diel variation was observed on some days during the summer (Fig. 4; examples of low and high diel variations in R_r during summer). Diel cycles of R_r were strongly related to temperature on all days (Pearson's r between temperature and $R_r = 0.64 \pm 0.03$). Q_{10} values calculated from diel patterns of R_r (hereafter 'apparent Q_{10}') were relatively low and constant in the winter, ranging from 1.07 to 5.0 with a mean of 3.0. However, apparent Q_{10} values calculated in this way for summer data were variable and extremely large, ranging from 2.5 to 104.8, with a mean of 24.2. The variation and magnitude of these values suggest that a process beyond simple temperature sensitivity was operating during the summer.

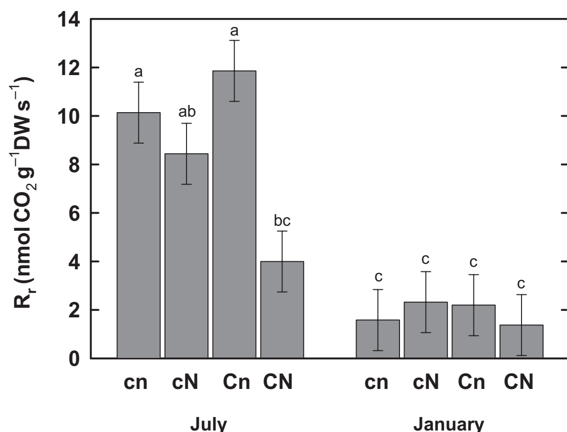

Figure 2. *In situ* respiration of attached loblolly pine fine roots. Treatments are: c, ambient [CO_2]; C, elevated [CO_2]; n, ambient nitrogen; N, nitrogen fertilized. Four subreplicates were averaged per plot ($n = 3$). Categories that do not share a letter are significantly different (Tukey adjusted $P < 0.05$). R_r was measured at ambient soil temperature: 20 °C in July and 10 °C in January.

We hypothesized that day-to-day variation in the diel pattern of R_r was influenced by substrate supply, as R_r responded to additions of exogenous glucose in the summer (Fig. 1). Therefore, we investigated the relationship between carbon assimilation [daytime NEE measured by eddy covariance, when photosynthetically active radiation (PAR) > 0] and the apparent Q_{10} calculated from diel cycles. Variation in the apparent Q_{10} was correlated with NEE (Fig. 5a; apparent $Q_{10} = 0.044 \times 0.0992{*}e^{(0.185 \times NEE)}$, $P < 0.01$, $r^2 = 0.83$). Furthermore, lag analysis indicated that recent carbon assimilation explained the observed apparent Q_{10} values; NEE from more than 1 d prior to measurements of R_r were not significantly correlated with apparent Q_{10} (Fig. 5b). It appears that NEE affected the temperature sensitivity of R_r instead of affecting R_r directly, as increasing NEE only slightly reduced the correlation between temperature and R_r (Pearson's r between temperature and $R_r = 0.726 - 0.005{*}NEE$, $P < 0.05$, $r^2 = 0.19$).

Yearly stand-level R_r

The yearly quantity of carbon respired by fine roots varied with elevated CO_2 and N fertilization (Fig. 6), and this variation was driven largely by the standing biomass of fine roots and tissue-specific rate of respiration. R_r released 645 ± 74 g C m^{-2} year^{-1} in ambient conditions, and this was not significantly affected by N fertilization alone (546 ± 74 g C m^{-2} year^{-1}, $P > 0.2$). Elevated CO_2 increased

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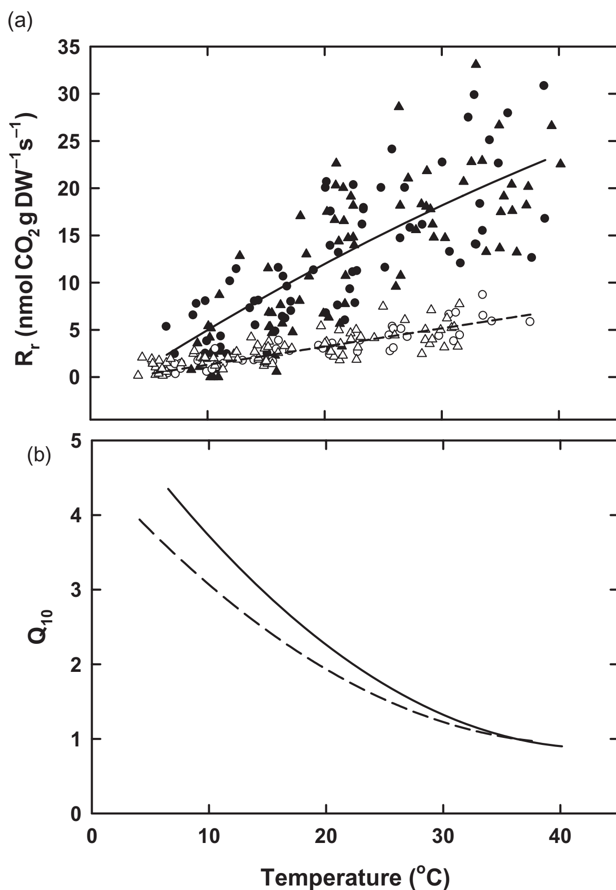

Figure 3. (a) Temperature sensitivity of fine-root respiration of adult loblolly pine trees. Summer data: solid symbols and line: $y = -7.25 + 1.17x - 0.011x^2$, $P < 0.01$, $r^2 = 0.61$). Winter data: open symbols and dashed line: $y = -0.0115 + 0.173x$, $P < 0.01$, $r^2 = 0.68$. Circles are ambient CO_2; triangles are elevated CO_2. Q_{10} values were generated from these data (b) using the following equation: $Q^{10} = 10^{(10 * \text{slope})}$. The slope was calculated as the derivative of the second-order polynomial describing $\log_{10}$ respiration versus temperature plots.

the amount of carbon released by R_r to 869.7 ± 74 g C m^{-2} year^{-1} ($P < 0.05$), primarily because of increased standing fine-root biomass in the elevated CO_2 plots. The combination of elevated CO_2 and N fertilization reduced R_r to 389 ± 73 g C m^{-2} year^{-1}, but this decrease was not statistically significant after the Tukey adjustment for multiple comparisons ($P > 0.05$). This reduction was driven by the 61% decrease in the tissue-specific R_r (Fig. 2) despite an increase in the standing root biomass relative to ambient conditions.

DISCUSSION

Fine-root respiration is a complex process best understood at multiple levels of organization in space and time. At the tissue level, instantaneous R_r was partially determined by substrate availability and ATP utilization (Fig. 1), and daily R_r was influenced by the temperature sensitivity of respiration, which was affected by recent canopy carbon assimilation (Figs 4 & 5). At the ecosystem scale, R_r was determined primarily by the standing crop of fine roots, which was likely governed by plant allocation to nutrient or water acquisition. R_r was reduced by the combination of elevated CO_2 and N fertilization (Fig. 2, trend in Fig. 6), but no changes in tissue chemistry (Table 1) or respiratory control (Fig. 1) were found that might explain this observation. This may be explained by increased above-ground net primary production (ANPP) in elevated CO_2 and N-fertilized plots, as ANPP has been shown to be inversely related to total below-ground allocation at this site (Palmroth *et al.* 2006). Perhaps less C is transported below-ground in these plots, reducing the C available for R_r.

The proximal limits to R_r varied seasonally but were not affected by elevated CO_2 or N fertilization. The limitation of R_r by enzyme capacity in the winter (Fig. 1) was likely caused by the reductions of enzyme activity in cold temperatures (Ryan 1991; Atkin, Edwards & Loveys 2000). Reduced respiratory capacity in the winter is consistent

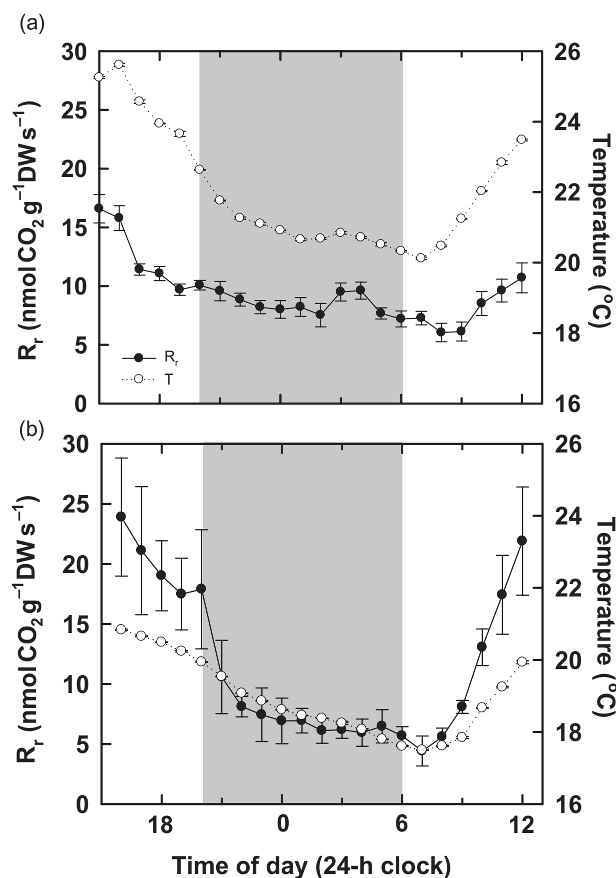

Figure 4. Examples of diel variation in loblolly pine fine-root respiration (R_r; ●, solid line) and temperature (○, dotted line). Each point indicates an hourly average of four subsamples, with three measurements per subsample. Light background indicates day; shaded background, night. The variation of R_r was small on some days [(a) 14 July 2006] with reasonable apparent Q_{10} values ($Q_{10} = 3.3$). R_r was highly variable on other days [(b) 5 July 2008] with very large apparent Q_{10} values ($Q_{10} = 79.6$).

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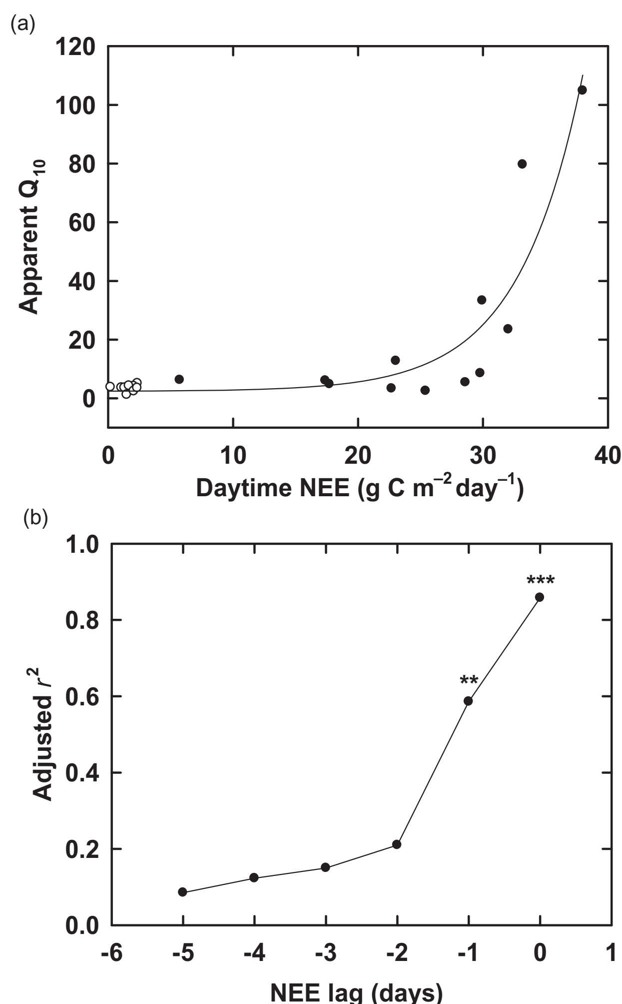

Figure 5. Relationship between apparent temperature sensitivity of fine-root respiration (Q_{10}) and simultaneous daytime net ecosystem exchange [NEE; (a)] Data are from diel gas exchange measurements as in Fig. 4 during July (●) and January (○). $y = 0.044* 0.0992*e$^$(0.185x)$ $P < 0.001$, $r^2 = 0.83$. (b) Regressions as in (a) fit using lagged daytime NEE values. ** indicates significance at $P < 0.01$; *** at $P < 0.001$.

with the significant reduction in fine root N (Table 1) and temperature sensitivity (Fig. 3a). This response is the opposite of temperature acclimation as it is generally understood (Atkin *et al.* 2005), suggesting that fine roots at this site enter a relatively dormant state during the winter (Alvarez-Uria & Korner 2007).

Yearly estimates of stand-level R_r were less sensitive to the temperature response function than expected. We recalculated yearly R_r assuming $Q_{10} = 2$ (George *et al.* 2003), and found that this overestimated R_r by 9.5%. Similarly, we found that applying the summer temperature response (Fig. 3a) to summer and winter tissue-specific rates overestimated R_r by only 0.7%. The reduced temperature sensitivity of R_r in the winter was thus unimportant to stand C balance at this site because the flux during these months was small, and soil temperatures were in a range where the temperature response functions converged (Fig. 2).

The combination of methods used in this study shed light on the mechanisms that caused previous estimates of R_r at this site to differ. Using the O_2 electrode method on roots obtained from soil cores, a method similar to that used in this study (Fig. 1), Matamala & Schlesinger (2000) estimated R_r to be 4.08 and 4.42 nmol CO_2 g^{-1} DW s^{-1} in ambient and elevated CO_2, respectively, whereas George *et al.* (2003), using gas exchange techniques on intact roots as in Fig. 2, estimated R_r to be 8.93 and 6.91 nmol CO_2 g^{-1} DW s^{-1} in ambient and elevated CO_2. These methods lead to estimates of annual R_r that varied by more than 100%. Although this could arise from interannual variation in R_r, results presented here suggested that much of the disparity is methodological. We estimated R_r using both methods in the same forest at the same time and found little correspondence (compare Fig. 1 and 2). A treatment effect of $CO_2 \times N$ was detected using *in situ* gas exchange (Fig. 1), but no treatment effects were detected using measurements of O_2 consumption (Fig. 2). It is possible that the damage response to excision and immersion in buffer for the O_2 consumption measurements overwhelmed the treatment differences. In addition, R_r measured by *in situ* gas exchange were higher than those of O_2 consumption in July, but the opposite occurred in January. These results highlight the disparate results obtained with different methods. Although both techniques disrupt the root–microbe–soil matrix, we believe the *in situ* method is more reflective of *in situ* fluxes as roots are left intact.

The yearly estimates of R_r reported here correspond with soil respiration (R_{soil}) measurements from this site. Compared with a 7 year mean of R_{soil} (Bernhardt *et al.* 2006), R_r as reported here comprised 43% of R_{soil} in ambient CO_2 and 50% of R_{soil} in elevated CO_2. These values are close to the average of 55% for all temperate coniferous forests, and are consistent with the trend of increasing R_r/R_{soil} with increasing R_{soil} (Subke, Inglima & Cotrufo 2006). N fertilization

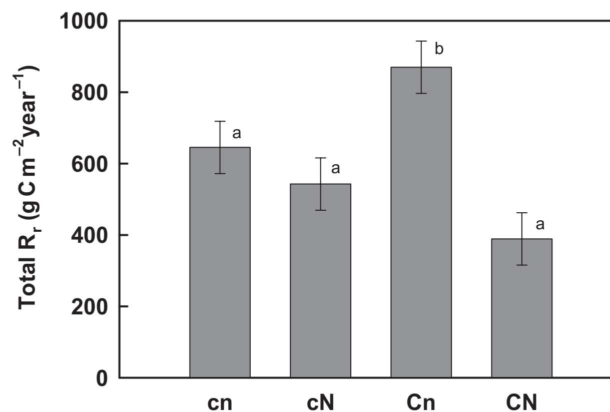

Figure 6. Total annual loss of carbon caused by fine-root respiration (R_r) in a loblolly pine forest exposed to elevated [CO_2] and nitrogen fertilization. Treatments are as follows: c, ambient [CO_2]; C, elevated [CO_2]; n, ambient nitrogen; N, nitrogen fertilized. Values are the mean of three experimental plots per treatment ($n = 3$). Treatments that do not share a letter are significantly different at $P < 0.05$ (Tukey adjusted P value).

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reduced R_{soil} by 20% when combined with elevated CO_2 but only 8.5% in ambient CO_2 (Oren *et al.* unpublished). The reduction of R_r by the combination of elevated CO_2 and N fertilization (Figs 2 & 6) could explain this 20% reduction in R_{soil}. Additionally, the observation that the Q_{10} of R_r declines with temperature (Fig. 3b) is supported by previous observations that the Q_{10} of R_{soil} declines with temperature at this site (Bernhardt *et al.* 2006). This correspondence with R_{soil} at this site increases our confidence in the accuracy of *in situ* gas exchange measurements of R_r.

The close correlation between NEE and the apparent Q_{10} of R_r (Fig. 4) suggests that the rate of root respiration is tightly and immediately coupled to canopy photosynthesis. Stoy *et al.* (2007) demonstrated a 1–3 d time lag between carbon uptake and R_{soil} in this forest, but overlapping lag times in the biological (plant and mycorrhizae) and physical (soil matrix) components in the ecosystem complicated efforts to definitively attribute this lag time to biotic or abiotic factors. Automated soil respiration measurements have documented temperature-independent diel cycles that follow light availability and photosynthesis in a deciduous forest (Liu *et al.* 2006) as well as an oak–grass savannah (Tang, Baldocchi & Xu 2005). Similarly, strong coupling between photosynthesis and R_{soil} has been observed in a *Pinus ponderosa* forest (Irvine, Law & Kurpius 2005). These studies and results from girdling experiments (e.g. Hogberg *et al.* 2001), suggest that there is a direct link between canopy photosynthesis and R_r. It is also possible that this coupling involved respiration by ectomycorrhizal fungi at the root surface, as it was not possible to separate mycorrhizae and fine roots without causing considerable damage. Thus, we are unable to determine if canopy photosynthesis is directly coupled with R_r, indirectly coupled to rhizosphere respiration via root exudation or both.

The coupling of canopy C assimilation and R_r suggests that elevated CO_2 should increase R_r by increasing canopy photosynthesis (Schafer *et al.* 2003), but we did not detect such an increase in R_r (Fig. 2). This is because we estimated *in situ* R_r using measurements in the morning from 0400 to 0700 h to minimize between-day variance in temperature; it is reasonable to expect that coupling with canopy photosynthesis was absent in these early morning hours. We lacked the sampling intensity to investigate treatment level variation in the NEE–R_r coupling. Future work on R_r could investigate the implications of the NEE–R_r coupling for yearly stand C balance.

The timescale of the observed coupling (1 d or less) is shorter than the 3–4 d lag between fixation and soil efflux inferred from isotope data in this forest (Andrews *et al.* 1999; Mortazavi *et al.* 2005). The longer lag times may reflect the physical lag associated with CO_2 movement through the soil before it is measured as surface efflux (Stoy *et al.* 2007). It is also possible that the process linking photosynthesis and R_r operates at a shorter timescale than actual carbohydrate transport between needles and fine roots. Models of phloem transport indicate that pressure-concentration waves propagate through a plant more quickly than the transport of individual sugar molecules (Thompson & Holbrook 2003; Thompson 2006). This indicates that high rates of photosynthesis could rapidly deliver sugars to distant tissues such as fine roots even if the delivered molecules were not fixed that day. Such an influx of sugar would likely stimulate R_r because of substrate limitation during the summer (Fig. 1).

We estimated the time it would take for sucrose loading into needle phloem at the top of the canopy to increase the sucrose concentration in the phloem of fine roots (propagation time: τ_p) using a theoretical model of phloem transport (Ferrier 1976; Thompson & Holbrook 2004) according to the equation $\tau_p = 0.5(\mu L^2 \Psi_\pi^{-1} k^{-1})$ where μ is viscosity, L is path length, Ψ_π is sap osmotic potential, and k is specific conductivity. We estimated μ to be 1.5e^{-9} MPa·s, Ψ_π to be 1.5 MPa (Thompson, personal communication), L to be 25 m (canopy height is 19 m), and k to be 4.4e^{-12} m^2 (Thompson & Holbrook 2003), leading to an estimate of 20 h for τ_p. This value is consistent with our results (Figs 4 & 5). Furthermore, varying μ, L, Ψ_π and k within reasonable limits lead to estimates of τ_p between 10 and 30 h, which is within the timeframe of the observed coupling (Fig. 5). Similarly, a maximum phloem transport rate on the order of 1 m h^{-1} (Peuke *et al.* 2001) results in similar lag times using the earlier mentioned assumptions.

CONCLUSIONS

Fine-root respiration is a complex process with controls that operate on different timescales and levels of organization. Elevated CO_2 and N fertilization did not alter the regulation of R_r, but elevated CO_2 increased stand-level R_r by increasing the amount of respiring tissue. The combination of elevated CO_2 and N showed a trend of reduced R_r. The mechanism for this is unknown, but could be caused by reduced C allocation below ground. Measurements of R_r with high temporal resolution detected a dynamic coupling between canopy C assimilation and the temperature dependence of R_r, suggesting that carbohydrate transport can increase ecosystem C loss on short timescales, although the effects of this coupling on stand C balance is not yet known. With further research it may be possible to predict rhizospheric respiration from eddy covariance measurements of ecosystem fluxes given accurate models of phloem wave propagation and mass transport.

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